

Quantification of Ant Attendance (Myrmecophily) of Lycaenid Larvae

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Abstract. A methodology is presented for quantifying ant attendance of lycaenid larvae. Attendance of larvae by *Formica pilicornis* Emery (Formicidae) is compared for 58 lycaenid species (primarily from California). The presence of larval myrmecophilous organs is compared with ant attendance rankings to estimate their relative contributions to attendance. Evidence is presented that dendritic setae may be as important in ant recruitment as the better known honey gland and eversible tubercle organs, and may be a primitive precursor of the latter.

Introduction

The family Lycaenidae is known for its larval myrmecophily and symbiotic ant-larval associations ranging from simple co-existence to parasitism have been described (see Hinton, 1951; Malicky, 1969a, b; Cottrell, 1984; Pierce, 1987; Maschwitz *et al.*, 1988, 1989). Perhaps the most common ant-larval interaction is mutualism, whereby larvae reward ants with a mixture of carbohydrates and amino acids (honey dew) in return for protection from predators. Malicky (1970) rejected mutualism in favor of ant appeasement as the dominant aspect of myrmecophily among lycaenids, largely because of lack of convincing evidence that ant attendance reduced larval predation. However, recent workers have provided evidence both of the nutritive value of larval secretions and the protective value of attending ants (Maschwitz, *et al.*, 1975; Pierce & Mead, 1981; Pierce & Eastal, 1986; Pierce, *et al.*, 1987; Fiedler & Maschwitz, 1988a; DeVries, 1988a, 1991a).

The diversity of ant-lycaenid relationships, combined with related morphological specializations, should provide valuable clues to lycaenid phylogeny. Indeed, Henning (1983a) updated Hinton's (1951) biological groups within the Lycaenidae (based on myrmecophily, carnivory, etc.) and noted their similarity to Eliot's (1973) proposed phylogeny, which is based primarily on adult morphology. Cottrell (1984) reviewed the complex diversity of lycaenid feeding strategies (e.g. aphytophagy has arisen independently at least eight times), and noted that attempts to

explain these strategies must be linked to a full appreciation of phylogeny. However, the apparent loss of myrmecophilous organs and myrmecophily in members of many lycaenid lineages and absence of convincing evidence of intermediate stages in the evolution of the various organs responsible for myrmecophily complicates the use of such features for determining phylogenetic relationships. For entire groups which lack certain organs, one can only speculate whether the group predates the evolutionary origin of those organs or if the organs were lost in the group's progenitor. Further, the anecdotal nature of most descriptions of myrmecophily allows only subjective qualitative comparisons among taxa.

In this paper a standardized testing procedure is presented to quantitatively compare a major aspect of myrmecophily, the degree of ant attendance of last instar lycaenid larvae. The degree of attendance is correlated with the presence of myrmecophilous organs (ant-organs): lenticles, eversible tubercles, honey gland(s) [respectively, perforated cupola organs, tentacular organs, and dorsal nectary organ and tentacular nectary organs of Cottrell (1984)], and dendritic setae (Ballmer & Pratt, 1989). The contribution of dendritic setae to myrmecophily, previously a matter of speculation (Fiedler & Maschwitz, 1988b; Ballmer & Pratt, 1989), is demonstrated by comparison of their densities with ant attendance rates among various *Lycaena* species, which lack all other known ant-organs except lenticles. Finally, the results of this study provide a basis for speculations on the sequence of origin of the various ant-organs.

Materials and Methods

The degree of ant attendance was measured as the mean number of seconds that larvae were attended per five minutes (300 s). For each five-minute observation period, one last instar larva was placed in a clear plastic arena (12 X 8.5 X 6 cm) containing five workers of *Formica pilicornis* Emery. Each observation period began ten seconds after initial ant-larval contact. For most species, observations were replicated at least ten times; the number of larvae tested per species ranged from 1 to 10. The number of seconds of attendance per five minutes was converted to percentage and then transformed to arc sine values for analysis.

Ant attendance primarily consisted of active antennation of the larval cuticle, as described by Malicky (1970), coupled with walking back and forth over the larva. Alarm behavior of ants in response to eversion of eversible tubercles (Malicky, 1969a; Claassens & Dickson, 1977; Fiedler & Maschwitz, 1988b) was also counted as attendance, although it usually resulted in briefly suspended (1-5 s) contact. Other behaviors that were not considered attendance include incidental contact (contact < 1 s), ants at rest or preening atop larvae (no antennation), and aggression. Instances of multiple simultaneous attendance were counted the same as single ant attendance.

Ants obtained from a wild *Formica pilicornis* colony near the community of Mountain Home (el. ca 1300 m), San Bernardino Co., CA, were used because they commonly tend lycaenid larvae in nature. Ants were collected as needed and

seldom kept for more than four days, since their reliability for larval attendance decreased over time in captivity. Ants that failed to attend larvae (which other ants did attend) usually died within a day. Therefore, control larvae of *Icaricia acmon* (Westwood & Hewitson) and *I. lupini* (Boisduval), which are commonly attended by *F. pilicornis* in nature, were used to gauge the attendance capacity of subject ants: Ants which demonstrated a reduced attendance of *I. acmon* or *I. lupini* (< 75% of normal attendance) (Table 1) were discarded. Ants were housed in 1 L plastic food containers and fed 10% honey water dispensed by a cotton wick. Lycaenid larvae were field-collected or reared from ova; 55 species were from Arizona and California; one species each came from Brazil, Thailand and the eastern United States.

A second experiment measured differences in ant attendance of *Plebulina emigdionis* (Grinnell) for two colonies of *F. pilicornis*. The ants were from Mountain Home and Victorville, CA, 57 km NW of Mountain Home. Ants from the latter site were found at the base of the host plant, *Atriplex canescens* (Pursh) Nutt, where the *P. emigdionis* larvae had been collected three months earlier. In order to remove bias due to possible differences in larval attractiveness, the same larvae were alternately exposed to ants from each site. This experiment was inspired by initial test observations that larvae were poorly attended by *F. pilicornis* (Mountain Home colony) in the laboratory even though they were strongly associated with the same ant species in the field.

Four species of *Lycaena* (*editha*, *heteronea*, *rubida*, and *xanthoides*) known to be myrmecophilous were compared with respect to ant attendance and the abundance of both dendritic setae and lenticles. This comparison also included one population of *L. heteronea* (Tioga Pass) which is apparently not myrmecophilous in nature.

Data were analyzed using ANOVA with Duncan's new multiple range test (statistical package adapted for personal computer by the UCR Statistics Department).

The nomenclature of higher taxonomic groups follows Eliot (1973), except that Riodinidae is treated as a subfamily of Lycaenidae.

Results

ANT-ATTENDANCE

Table 1 lists all species tested in decreasing order of their mean measured ant attendance. These are grouped in 13 sets whose members' attendance means are not significantly different ($P < 0.01$). Generally, these sets broadly overlap; however, there is very little overlap between sets of means greater than 50% (sets A-G) and those less than 50% (sets I-M).

Table 2 lists the tested species according to natural groups and compares them with respect to mean ant attendance, the presence of ant-organs, and observed myrmecophily in nature. All species have cuticular lenticles; additional ant-organs are absent in two Riodininae, one Miletinae, 7 Lycaeninae, and two Theclini. A honey gland is present in all 22 Polyommatainae and all 19 Theclinae. Tentacle nectary organs are present in one riodinine. Eversible tubercles occur in one thecline (*F. fulgida*) and all polyommataines except *P. speciosa*. Dendritic setae occur in one riodinine, 4 lycaenines, 15 theclines, and 20 polyommataines.

Table 1. Larval attendance by *Formica pilicornis*

Species	N	Mean ¹	Duncan's Test
<i>Lycaena editha</i> (Mead)	12	100	A
<i>Phaeostrymon alcestis</i> (W. H. Edwards)	10	100	A
<i>Satyrrium auretteum</i> (Boisduval)	10	100	A
<i>Satyrrium behrii</i> (W. H. Edwards)	10	100	A
<i>Satyrrium californicum</i> (W. H. Edwards)	10	100	A
<i>Satyrrium saepium</i> (Boisduval)	10	100	A
<i>Satyrrium sylvinum</i> (Boisduval)	10	100	A
<i>Icaricia lupini</i> (Boisduval)	11	100	A
<i>Flos fulgida</i> (Hewitson)	13	99	A
<i>Everes comyntas</i> (Godart)	10	99	A
<i>Harknclenus titus</i> (Fabricius)	10	99	A
<i>Philotes sonorensis</i> (C. & R. Felder)	10	99	A
<i>Glaucopsyche lygdamus</i> (Doubleday)	10	99	A
<i>Hemiargus isola</i> (Reakirt)	10	98	A
<i>Eurybia</i> sp.	10	98	A
<i>Celastrina argiolus</i> (L.)	10	97	A
<i>Glaucopsyche piasus</i> (Boisduval)	10	96	A
<i>Lycaena rubida</i> (Behr)	10	96	AB
<i>Brephidium exile</i> (Boisduval)	10	92	ABC
<i>Icaricia icarioides</i> (Boisduval)	10	91	ABCD
<i>Icaricia acmon</i> (Westwood & Hewitson)	11	89	ABCDE
<i>Hemiargus ceraunus</i> (Fabricius)	10	87	ABCDE
<i>Everes amyntula</i> (Godart)	10	86	ABCDE
<i>Strymon melinus</i> (Hübner)	10	86	ABCDE
<i>Lycaena xanthoides</i> (Boisduval)	21	85	ABCDE
<i>Euphilotes pallescens elvirae</i> (Mattoni)	10	82	ABCDEF
<i>Satyrrium tetra</i> (W. H. Edwards)	10	82	ABCDEFG
<i>Lycaeides melissa</i> (W. H. Edwards)	20	76	BCDEFG
<i>Incisalia mossii</i> (Hy. Edwards)	10	72	BCDEFG
<i>Icaricia shasta</i> (W. H. Edwards)	21	70	BCDEFG
<i>Incisalia augustinus</i> (W. Kirby)	12	69	CDEFG
<i>Icaricia neurona</i> (Skinner)	11	68	DEFG
<i>Lycaeides idas</i> (L.)	10	68	DEFG
<i>Mitoura spinetorum</i> (Hewitson)	10	66	DEFG
<i>Fixsenia ontario</i> (W. H. Edwards)	11	65	EFG
<i>Satyrrium fuliginosum</i> (W. H. Edwards)	10	64	FGH
<i>Lycaena heteronea</i> (Boisduval) (W) ²	10	63	GH
<i>Lycaena mariposa</i> (Reakirt)	10	41	HI
<i>Lycaena arota</i> (Boisduval)	10	28	IJ
<i>Mitoura loki</i> (Skinner)	10	26	IJK
<i>Callophrys perplexa</i> (Barnes & Benjamin)	7	24	IJK
<i>Plebulina emigdionis</i> (Grinnell)	20	16	IJKL

<i>Calephelis wrighti</i> Holland	11	18	IJKL
<i>Euphilotes mojave</i> (Watson & Comstock)	10	18	IJKLM
<i>Habrodais grunus</i> (Boisduval)	10	13	IJKLM
<i>Lycaena heteronea</i> (Boisduval) (T) ³	10	12	IJKLM
<i>Hypaurotis crysalus</i> (W. H. Edwards)	10	11	IJKLM
<i>Mitoura siva</i> (W. H. Edwards)	10	11	JKLM
<i>Leptotes marina</i> (Reakirt)	10	9	JKLM
<i>Lycaena phlaeas</i> (L.)	10	7	JKLM
<i>Lycaena hermes</i> (W. H. Edwards)	13	7	JKLM
<i>Lycaena gorgon</i> (Boisduval)	10	5	KLM
<i>Feniseca tarquinius</i> (Fabricius)	10	5	KLM
<i>Philotiella speciosa</i> (Hy. Edwards)	10	4	LM
<i>Apodemia mormo</i> (C & R. Felder)	7	4	LM
<i>Lycaena nivalis</i> (Boisduval)	5	3	LM
<i>Euphilotes bernardino martini</i> (Mattoni)	7	2	LM
<i>Lycaena cuprea</i> (W. H. Edwards)	10	0	M
<i>Atlides halesus</i> (Cramer)	12	0	M

¹ Rounded mean percent time of ant attendance per 300 s; means followed by same letter are not significantly different (P < 0.01) according to Duncan's new multiple range test.

² W = unnamed subspecies from Warren Canyon, Mono Co., Ca.

³ T = unnamed subspecies from Tioga Pass, Mono Co., Ca.

Table 2. Ant attendance and myrmecophilous organs of some lycaenid larvae

Subfamily ¹	Species	AA ²	HG ³	TNO	ET	L	DS	M
Riodininae								
Riodinini								
	<i>A. Mormo</i>	4	—	—	—	+	—	—
	<i>C. wrighti</i>	18	—	—	—	+	—	—
	<i>Eurybia</i> sp.	98	—	+	—	+	+	+
Miletinae								
Miletini								
	<i>F. tarquinius</i>	5	—	—	—	+	—	—
Lycaeninae								
Lycaenini								
	<i>L. cuprea</i>	0	—	—	—	+	—	—
	<i>L. phlaeas</i>	7	—	—	—	+	—	—
	<i>L. arota</i>	28	—	—	—	+	—	?
	<i>L. hermes</i>	7	—	—	—	+	—	?
	<i>L. mariposa</i>	41	—	—	—	+	—	?
	<i>L. nivalis</i>	3	—	—	—	+	—	?
	<i>L. gorgon</i>	5	—	—	—	+	—	—

<i>L. heteronea</i> (W) ⁴	63	—	—	—	+	+	+
<i>L. heteronea</i> (T) ⁵	12	—	—	—	+	+	—
<i>L. editha</i>	100	—	—	—	+	+	+
<i>L. rubida</i>	96	—	—	—	+	+	+
<i>L. xanthoides</i>	85	—	—	—	+	+	+

Theclinae

Theclini

<i>H. grunus</i>	13	—	—	—	+	—	—
<i>H. crysalus</i>	11	—	—	—	+	—	?

Eumaeini

<i>S. melinus</i>	86	+	—	—	+	+	+
<i>C. perplexa</i>	24	+	—	—	+	+	—
<i>I. augustinus</i>	69	+	—	—	+	+	?
<i>I. mossii</i>	72	+	—	—	+	+	+
<i>M. spinetorum</i>	66	+	—	—	+	+	—
<i>M. loki</i>	26	+	—	—	+	—	?
<i>M. siva</i>	11	+	—	—	+	—	?
<i>A. halesus</i>	0	+	—	—	+	—	—
<i>P. alcestis</i>	100	+	—	—	+	+	?
<i>H. titus</i>	99	+	—	—	+	+	+
<i>F. ontario</i>	65	+	—	—	+	+	?
<i>S. auretteorum</i>	100	+	—	—	+	+	?
<i>S. behrii</i>	100	+	—	—	+	+	?
<i>S. californicum</i>	100	+	—	—	+	+	?
<i>S. fuliginosum</i>	64	+	—	—	+	+	+
<i>S. saepium</i>	100	+	—	—	+	+	+
<i>S. sylvinum</i>	100	+	—	—	+	+	?
<i>S. tetra</i>	82	+	—	—	+	+	?

Arhopalini

<i>F. fulgida</i>	99	+	—	+	+	—	+
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Polyommatainae

Polyommataini

<i>B. exile</i>	92	+	—	+	+	—	+
<i>L. marina</i>	9	+	—	+	+	+	+
<i>C. argiolus</i>	97	+	—	+	+	+	+
<i>P. sonorensis</i>	99	+	—	+	+	+	+
<i>P. speciosa</i>	4	+	—	—	+	+	—
<i>E. bernardino martini</i>	2	+	—	+	+	+	+
<i>E. enoptes mojave</i>	18	+	—	+	+	+	+
<i>E. pallescens elvirae</i>	82	+	—	+	+	+	+
<i>G. lygdamus</i>	99	+	—	+	+	+	+
<i>G. piasus</i>	96	+	—	+	+	+	+

<i>H. ceraunus</i>	87	+	—	+	+	+	+
<i>H. isola</i>	98	+	—	+	+	—	?
<i>I. acmon</i>	89	+	—	+	+	+	+
<i>I. icarioides</i>	91	+	—	+	+	+	?
<i>I. lupini</i>	100	+	—	+	+	+	+
<i>I. neurona</i>	68	+	—	+	+	+	?
<i>I. shasta</i>	70	+	—	+	+	+	+
<i>P. emigdionis</i>	16	+	—	+	+	+	+
<i>L. idas</i>	68	+	—	+	+	+	?
<i>L. melissa</i>	76	+	—	+	+	+	+
<i>E. amyntula</i>	86	+	—	+	+	+	+
<i>E. comyntas</i>	99	+	—	+	+	+	+

- 1 Taxa grouped according to subfamily, tribe, and lower category relationships.
- 2 Rounded mean percent ant attendance per 300 s by *Formica pilicornis*.
- 3 HG = honey gland, TNO = tentacle nectary organs, ET = eversible tubercles, L = lenticles, DS = dendritic setae, M = myrmecophily in nature; + = present, <197> = absent, ? = undetermined.
- 4 W = unnamed subspecies from Warren Canyon, Mono Co., Ca.
- 5 T = unnamed subspecies from Tioga Pass, Mono Co., Ca.

Although greater mean ant attendance roughly coincides with presence of more ant-organs, there are notable exceptions. Of the 27 most strongly attended species (Table 1, set A), 11 have four types of ant-organs, but the remainder have only two or three. And while 9 of the 16 least attended species (Table 1, set M) lack any ant-organs other than lenticles, 3 others have four types of ant-organs. Similar exceptions occur within subfamilial and tribal lineages.

Myrmecophily has not been systematically investigated among the lycaenid species addressed in this study. Table 4 tabulates the previously unreported ant-lycaenid relationships observed in the course of a survey of the California lycaenid fauna (Ballmer & Pratt, 1989). Table 1 indicates the known status of myrmecophily for all test species compiled from field observations (Ballmer & Pratt, personal observations; D. M. Wright, personal communication). Nearly all of the species known to be myrmecophilous in nature are in the Polyommatainae; four lycaenines, five theclines, and one riodinine are also known to be myrmecophilous. This tabulation is biased by the nature of the larval collection techniques. The larval hosts of most of the lycaenines and polyommataines are low shrubs and herbs on which larvae were most easily located by searching; in these species, ant associations were easily observed and aided in locating larvae. Because many of the theclines were collected by beating foliage or reared from ova in the lab, observations of natural ant associations were precluded for most species. The high degree of experimental ant attendance observed here suggests that some Eumaeini may also be attended by ants in nature.

ABUNDANCE OF DENDRITIC SETAE AND LENTICLES AMONG *LYCAENA* SPECIES

Table 3 compares the abundance of dendritic setae and lenticles on larvae of the four *Lycaena* species which were most ant-attended. All four species have also been found attended by ants in the field (Ballmer & Pratt, 1989, and personal observations). However, the population of *L. heteronea* from Tioga Pass had the fewest dendritic setae and lenticles and was also least ant-attended; coincidentally, when these larvae were found in the field, no ants were in attendance. Because of variability in abundance of lenticles and dendritic setae among individual larvae of these populations, and because other larvae were used (in some cases) to generate ant attendance values (Tables 1, 2), the former character data cannot validly be correlated with the latter. Nevertheless, the data suggest that abundance of both lenticles and dendritic setae may be positively related to ant attendance. Of these, the abundance of dendritic setae seems to be more strongly associated with attendance.

COLONY-RELATED DIFFERENCES IN ANT ATTENDANCE

Comparison of attendance of *P. emigdionis* by *F. pilicornis* from two locations reveals significant differences. Larvae were tended very little by ants from Mountain Home (Table 1), but significantly more ($P = 5 \times 10^{-15}$) by ants from Victorville (mean percent attendance = 75, $N = 18$).

NON-TENDING ANT BEHAVIOR

Non-tending ant responses to larvae during tests included indifference and aggression. The majority of ants not attending larvae were indifferent to them. When these ants encountered larvae, they generally investigated them briefly with minimal antennation and then moved on. The infrequent instances of aggression were usually initiated immediately upon contact by a single ant and lasted for 2-10 s. Invariably, the point of attack was just ventral to the lateral fold of the larva. Larvae of *A. mormo* and *L. arota* were attacked for periods of 90 s, and one larva of

Table 3. Dendritic setae and lenticles on myrmecophilous *Lycaena* larvae

species	N	Dendritic Setae ¹	Lenticles ¹
<i>L. rubidus</i>	10	171 ± 41 A	993 ± 89 A
<i>L. editha</i>	4	140 ± 20 A	909 ± 67 A
<i>L. xanthoides</i>	4	84 ± 8 B	761 ± 83 B
<i>L. heteronea</i> (W) ²	7	73 ± 44 B	679 ± 123 B
<i>L. heteronea</i> (T) ³	4	11 ± 8 C	646 ± 70 B

¹ Mean totals ± standard deviation; means followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's new multiple range test.

² Population from Warren Canyon, Mono Co., California.

³ Population from Tioga Pass, Mono Co., California.

Table 4. Ants associated with lycaenid larvae in California

Attending ant species ¹	Lycaenid species
<i>Aphaenogaster occidentalis</i> Emery	<i>Icaricia acmon</i>
<i>Camponotus essigi</i> M. Smith	<i>Euphilotes pallescens elvirae</i>
<i>Camponotus vicinus</i> Mayr	<i>Satyrium fuliginosum</i>
<i>Conomyrma bicolor</i> (Wheeler)	<i>Everes amyntula</i>
<i>Conomyrma</i> sp.	<i>Glaucopsyche piasus</i>
<i>Crematogaster californica</i> Emery	<i>Euphilotes enoptes smithi</i>
<i>Crematogaster mormonum</i> Emery	<i>Philotes sonorensis</i>
<i>Forelius pruinus</i> (Roger)	<i>Euphilotes pallescens elvirae</i>
	<i>Hemiargus ceraunus</i>
<i>Formica altipetens</i> Wheeler	<i>Lycaena editha</i>
<i>Formica lasioides</i> Emery	<i>Icaricia acmon</i>
<i>Formica moki</i> Wheeler	<i>Euphilotes battoides comstocki</i>
<i>Formica neoclara</i> Emery	<i>Glaucopsyche lygdamus</i>
<i>Formica neogagates</i> Emery	<i>Lycaeides melissa</i>
<i>Formica obscuripes</i> Forel	<i>Everes amyntula</i>
<i>Formica pilicornis</i> Emery	<i>Icaricia acmon</i> , <i>Icaricia lupini</i> , <i>Glaucopsyche piasus</i> , <i>Lycaena heteronea</i> , <i>Lycaena xanthoides</i> , <i>Plebulina emigdionis</i>
<i>Formica subsericea</i> Say	<i>Euphilotes battoides battoides</i>
<i>Formica</i> sp. (<i>fusca</i> group)	<i>Euphilotes battoides battoides</i> , <i>E. b. comstocki</i> , <i>Everes amyntula</i> , <i>Icaricia acmon</i>
<i>Formica</i> sp. (<i>microgyna</i> group)	<i>Satyrium fuliginosum</i>
<i>Formica</i> sp. (<i>rufa</i> group)	<i>Satyrium fuliginosum</i>
<i>Iridomyrmex humilis</i> (Mayr)	<i>Euphilotes bernardino bernardino</i> , <i>Icaricia acmon</i> , <i>Leptotes marina</i> , <i>Strymon melinus</i>
<i>Lasius niger</i> (L.)	<i>Everes amyntula</i>
<i>Lasius pallitarsus</i> (Provancher)	<i>Euphilotes battoides battoides</i>
<i>Monomorium</i> sp.	<i>Euphilotes battoides comstocki</i>
<i>Myrmecocystus mimicus</i> Wheeler	<i>Euphilotes pallescens elvirae</i>
<i>Myrmecocystus semirufus</i> Emery	<i>Glaucopsyche lygdamus</i>
<i>Tapinoma sessile</i> (Say)	<i>Euphilotes enoptes ancilla</i> , <i>E. e. smithi</i> , <i>Glaucopsyche lygdamus</i>

¹ Records based on the authors' field observations.

A. halesus (Cramer) was bitten continuously for 60 min, resulting in perforation of the larval cuticle with loss of hemolymph.

When bitten, larvae usually remained motionless, sometimes after curling into a 'C' shape. However, larvae of *A. mormo* thrashed about and regurgitated a fluid which, upon contact, caused ants to immediately withdraw and preen themselves. This behavior may be similar to the "beat reflex" observed in *Hamearis lucina* (L.) by Malicky (1969a, 1970).

Another defense against ants occurs in the Neotropical riordinine *Sarotagyas* (Cramer), whose larvae are densely covered with tufts of long, slender, easily-broken setae; ants which contact the setae spend much time cleaning their antennae and avoid further contact (DeVries, 1988b). Larvae of *C. wrighti*, which are similarly covered with tufts of long, slender, fragile setae (Ballmer & Pratt, 1989), did not induce such a repellent response in *F. pilicornis*; as with other poorly attended species, ants generally ignored *C. wrighti* after initial investigation.

Discussion

EXPERIMENTAL VS. NATURAL ANT ATTENDANCE

The ant attendance rankings reported here are not considered equivalent to the relative degree of myrmecophily for the larvae in nature. Many of the lycaenids tested have been found naturally in association with two or more ant species (Table 4), as well as without any attending ants (Ballmer & Pratt, personal observations). Even though myrmecophily is facultative in most California lycaenids (Ballmer & Pratt, personal observations) and most of the North American fauna (Pierce, 1987), this condition does not preclude differences in relative myrmecophily for different ant-lycaenid species combinations. Such a situation does exist with *L. marina*, which was well attended by *I. humilis* in the field but was poorly attended by *F. pilicornis* in the lab. Additionally, various environmental factors could affect ant attendance under more natural conditions (e.g. access to the nest and nest-mates, other nutrition sources, stress levels, etc).

Intraspecific geographic differences can occur for ant-larval interaction; this dictates caution in applying the test results to other populations. For example, two populations of *L. heteronea* differed significantly in attractiveness to the same ants, and, vice versa, larval attendance differed significantly for two populations of the ant, *F. pilicornis* with respect to *P. emigdionis*.

SOURCES OF EXPERIMENTAL ERROR

The design of this experiment could have contributed to erroneous estimates of ant attendance values. Because single and multiple simultaneous attendance were counted equally, real differences among the more highly attended species could not be distinguished; this problem might be overcome by counting all attending ants separately. Also, because many ants initially palpate and investigate any new object in their environment, the relatively short exposure times may have resulted in somewhat inflated attendance values for some species. However, the 10 s delay between initial ant contact and the onset of recording attendance at least partially compensated for this behavior. It is also possible that greater attendance values might have been obtained with longer exposure times through nest-mate recruitment, as demonstrated for the ant, *Tetramorium caespitum* (L.), attending larvae of *Polyommatus coridon* (Poda) (Fiedler & Maschwitz, 1989).

In spite of the limitations described, this test procedure provides a simple, repeatable means of measuring ant attendance of larvae under uniform conditions.

Lenticles

Lenticles are low-relief cuticular features for which a putative chemical communication function (Hinton, 1951; Malicky, 1969a, 1970; Henning 1983b) may require direct contact with ant antennal receptors. Indeed, antennation of larval cuticle is an important feature in ant attendance in this study and others (Malicky, 1969a, 1970; Fiedler & Maschwitz, 1989), and is often most intense in regions where lenticles are concentrated. In these areas ant-lenticle contact may be facilitated by the presence of generally sparser and/or shorter setae than occur elsewhere (but see dendritic setae, below).

In this study there is no evidence that lenticles contributed to ant attendance. All species with greater than 50% attendance (Table 1, sets A-G), possess additional ant-organs, whereas 12 of the 22 species having less than 50% attendance (Table 1, sets I-M) do not. Nevertheless, significant differences in attendance observed among the 12 species having only lenticle ant-organs may be due to quantitative and/or qualitative differences in semiochemicals associated with the lenticles.

Among populations of myrmecophilous *Lycaena* species, the ranked order of ant attendance (Table 1) corresponds to the ranked order of mean abundance of both lenticles and dendritic setae (Table 3). Of these, the abundance of dendritic setae is a better indicator of attendance. While the essentially non-myrmecophilous *L. heteronea* population from Tioga Pass had significantly less attendance and significantly fewer dendritic setae than the other populations, it did not have significantly fewer lenticles than *L. heteronea* from Warren Canyon and *L. xanthoides*.

In spite of accumulated evidence of ant adoption substances in or on the surface of lycaenid larvae (Malicky, 1969b, 1970; Henning, 1983b), the contribution of lenticles to myrmecophily remains obscure. As noted by Cottrell (1984), a number of different compounds may be involved and at least some may arise from cuticular sources other than lenticles. Such sources may include the cuticle itself, specialized setae, or the putative dermal glands (indicated by some surface pores and deep pits) found on lycaenid larvae (Wright, 1983; Kitching & Luke, 1985; DeVries et al, 1986; Kitching, 1987). Also, DeVries (1991b) noted that the evolutionary origin of lenticles may be unrelated to myrmecophily, since they occur in non-myrmecophilous hesperiids, as well as in both myrmecophilous and non-myrmecophilous lycaenids.

Honey gland

Aside from lenticles, the honey gland is the most prevalent ant-organ among the species tested. The function of this organ in ant recruitment may be largely, if not solely, related to the secretion of liquid nutrition.

Many ants which specialize in collecting the sugary fluids produced by homopterans and plant nectaries also attend lycaenid larvae (DeVries, 1991a, b). The honey gland is therefore a broad-spectrum ant-attractor, as opposed to other ant-organs which may produce more specific semiochemical attractants. The attractiveness of the honey gland was quantified by Fiedler & Maschwitz (1989) for *Polyommatus coridon* (Poda) by comparing ant recruitment for larvae with functional and blocked (resin-covered) honey glands. It may be more difficult to quantify the nutritional component of attractiveness of this organ among species tested here due to possible additive attractiveness of concentrations of lenticles and dendritic setae occurring along its margins. Although not quantified here, the time spent in feeding from the honey gland was usually relatively small compared to total attendance time.

The honey gland occurs widely in the Polyommatinae and Theclinae, but is apparently absent in other lycaenid subfamilies (Cottrell, 1984). Kitching and Luke (1985) refer to the condition in which a honey gland is absent as myrmecoxeny, and note that the condition is imperfectly correlated with lack of ant attendance. The strong ant attendance of some *Lycaena* species reported here illustrates that myrmecophily and myrmecoxeny are not mutually exclusive. Furthermore, although ants were observed to imbibe fluid from the honey gland of polyommataine larvae, such secretions were not observed in any thecline larva in the tribe Eumaeini. The latter are apparently functional myrmecoxenes.

Tentacle nectary organs

Tentacle nectary organs occur only in Riodininae and combine the general appearance, size, and location (posterior to A-8 spiracles) of eversible tubercles with the secretory function of the dorsal honey gland of Polyommatinae and Theclinae. Although Cottrell (1984) noted that tentacle nectary organs differ from eversible tubercles in producing visible secretions, and in lacking terminal setae, he presumed these organs to be homologous. DeVries (1991b), however, considered the ant-organs of riodinines to be analogous, not homologous, to the ant-organs of other lycaenid groups. Nevertheless, there are convergent exceptions to the usual physical distinctions between tentacle nectary organs and eversible tubercles. Thus, the eversible tubercles of some non-riodinine lycaenids, e.g. the Australian *Candalides xanthospilos* (Hübner) (Polyommatinae) and *Ogyris genoveva* Hewitson (Theclinae), lack terminal setae (Ballmer & Pratt, 1989), while the tentacle nectary organs of at least one myrmecophilous riodinine (*Setabis* sp.) apparently do not produce a liquid secretion (Ballmer, personal observations).

Eversible tubercles

The most notable effect of the eversible tubercles was the apparent induction of heightened activity and aggressive posturing in attending ants. When eversible tubercles (segment A-8) were briefly everted (< 1

s), attending ants usually dashed about with open mandibles, as though alerted to danger. This phenomenon was typical in those species having eversible tubercles. Such heightened ant activity (alarm behavior) following eversion of the tubercles could drive away predators and is probably commonplace in the Lycaenidae (Claassens & Dickson, 1977; Henning, 1983b; Cottrell, 1984; DeVries, 1984; Kitching & Luke, 1985; Fiedler & Maschwitz, 1988b), although Malicky (1969a, 1970) failed to observe such behavior. Even non-excited ants walking on larvae may inhibit attack by predators; K. Calloway (*in litt*) has found that non-attendant movement of ants on the hostplant near larvae of *Brephidium exile* (Boisduval) can effectively 'scare' off predators. Larvae of *P. emigdionis* were exceptional in that they everted their tubercles more frequently (especially while crawling) and for longer duration (often several seconds) than did other species, yet alarm behavior by attending ants was infrequent and generally less intense than with other species.

The mechanism by which the eversible tubercles induce heightened activity in ants is generally believed to be through release of chemicals which mimic ant alarm pheromone(s). Perhaps the most convincing evidence for this is provided by Henning (1983b), who showed that an extract of larval cuticle containing eversible tubercles of *Aloeides dentatis* (Swierstra) elicited initial alarm reaction and subsequent attraction in *Acantholepis capensis* Mayr. The anterior tentacle organs of some riordinines appear to have a similar function (DeVries, 1988a).

Eversible tubercles are somewhat more wide spread than the dorsal honey gland in the Lycaenidae. In addition to frequent occurrence among theclines and polyommattines, they also occur in the Curetinae and Liphyrinae (Cottrell, 1984).

Dendritic setae

The dendritic setae occurring on larvae and/or pupae of many members of the Riordininae, Lycaeninae, Polyommattinae, and Theclinae (Ross, 1964; Lawrence & Downey, 1966; Schremmer, 1978; Kitching, 1983; Kitching & Luke, 1985; Fiedler, 1988; Pratt, 1988; Ballmer & Pratt, 1989, and unpublished observations) may release semiochemicals which affect ant behavior (Fiedler & Maschwitz, 1988b). Although varying in density and distribution among species, dendritic setae tend to be concentrated in many of the same areas as lenticles, especially around the honey gland and spiracles. They are usually longer, more erect, and have longer lateral processes than surrounding setae (see Ballmer & Pratt, 1989); their greater flexibility and reduced pigmentation also suggest a thinner setal wall which could facilitate dissemination of volatile compounds. The generally greater prominence of dendritic setae probably also facilitates their contact with the antennae of attending ants.

Among the species tested here, the presence of dendritic setae appears to be a better indicator of ant attendance than is the presence of other ant-organs. They were present in all theclines (except *F. fulgida*) having

greater than 50% attendance and absent in all theclines (except *C. perplexa*) having less than 50% attendance. The relationship of dendritic setae to ant attendance among polyommattines is less clear; the two species lacking dendritic setae, *B. exilis* and *H. isola*, were strongly attended.

The relationship of dendritic setae to ant attendance is most apparent in the Lycaeninae, a group that lacks both honey gland and eversible tubercles. The four species of *Lycaena* found to have dendritic setae were also the most strongly attended (Table 2). Among these four species, greater abundance of dendritic setae (Table 3) coincides with greater ant attendance (Table 1). Two populations of *L. heteronea*, representing two subspecies (Emmel & Pratt, in press) from the eastern central Sierra Nevada illustrate infraspecific variations in both abundance of dendritic setae and ant attendance.

If dendritic setae are homologous with the spiculate setae of eversible tubercles and anterior tentacle organs, then the latter organs may be viewed as specializations of the former to accumulate (while retracted) and disseminate (when everted) a higher concentration of semiochemicals. Since ant pheromones which act as attractants at low concentrations may incite alarm behavior at higher concentrations (Blum, 1974; Henning, 1983b), a constant release of semiochemicals by exposed dendritic setae might yield concentrations sufficient to attract ants, while higher concentrations sufficient to alarm them might be disseminated when tubercles are infrequently and momentarily everted.

The frequent and prolonged eversion of the tubercles observed for *P. emigdionis* might result in a relatively constant low level of release of semiochemicals similar to that hypothesized for exposed dendritic setae. This might explain why little alarm behavior was elicited by the eversion of tubercles by larvae of this species. The frequent and prolonged eversion of the tubercles could be important in maintaining a retinue of attending ants while larvae periodically move between subterranean diurnal resting sites and above-ground nocturnal foraging sites (Ballmer & Pratt, personal observations). In an apparently similar behavior, larvae of *Aloeides thyra* (L.) and *A. dentatis* (Swierstra) are reported to repeatedly and rapidly evert their tubercles when leaving their diurnal ant nest shelters to feed nocturnally on vegetation (Claassens & Dickson, 1977; Henning 1983a, b).

COLONY-SPECIFIC ATTENDANCE RATES

An example of colony-specific differences in ant attendance is apparent with *P. emigdionis*. Perhaps the more attendant group of ants had 'learned' to associate with *P. emigdionis* through long-term contact in the field, or may also represent a cryptic sibling species or ecotype adapted to *P. emigdionis*. The patchy distribution of this lycaenid (Emmel & Emmel, 1973) and its occurrence in small dense colonies, often within larger stands of host plant (Ballmer & Pratt, personal observations) may

be due to a special relationship between the butterfly and its attendant ants. This is reinforced by observations that field-collected larvae were nearly always found with *F. pilicornis* in diurnal shelters at the plant base (below soil surface) or while feeding crepuscularly on above-ground foliage.

NONSPECIFIC MYRMECOPHILY

In contrast to *P. emigdionis*, which was highly attended only by a particular colony (or variety) of *F. pilicornis* from the larval collection site, some lycaenids were highly attended in spite of their origin outside the ant's range of distribution. *Flos fulgida* from Southeast Asia (Chiang Mai, Thailand) was highly attractive to *F. pilicornis*. Twenty larvae of *F. fulgida* were found in nature individually accompanied by numerous (often more than twenty) ants (*Hypoclinea* sp.) in folded leaf shelters. Although that situation may signal a species-specific relationship, the presence of a more generalized myrmecophilic factor in *F. fulgida* is indicated by its attractiveness to *F. pilicornis*.

THE ORIGINAL ANT ORGAN?

Knowledge of the sequence of origin of the various ant-organs could be helpful in understanding the evolutionary history of the Lycaenidae. Although it has been speculated that a nutritional secretory organ, such as the dorsal honey gland, was a feature of the primitive lycaenid ancestor (Hinton, 1951; Malicky, 1970; Pierce, 1987), cuticular lenticles, which occur much more widely (Hinton, 1951; Malicky, 1969a; Ballmer & Pratt, 1989), were probably derived earlier. Lenticles are the only ant-organs that occur in all lycaenid subfamilies for which larvae have been examined. Dendritic setae and their possible derivatives, eversible tubercles and anterior tentacle organs, also may be more widespread and potentially of earlier origin than nectary organs.

Whereas nutritive secretions from a honey gland or tentacle nectary organs may attract and bind a broad spectrum of fluid-foraging ant species, semiochemicals released by lenticles, dendritic setae, eversible tubercles, and anterior tentacle organs might achieve a similar attraction or bonding with less energy expenditure. Of these organs, dendritic setae may offer the best combination of structural simplicity, energy efficiency and effectiveness in disseminating ant-attracting semiochemicals. Such semiochemicals might also facilitate more specific and more efficient symbiotic relationships than nectary gland secretions.

The similarity of dendritic setae to spiculate setae typically found on eversible tubercles and anterior tentacle organs, in both structure and apparent function, suggests that the latter organs may be specialized derivatives of the former. Such a derivation would strengthen Fiedler's (1988) hypothesis that the Lycaeninae is an ancient group predating the origins of the closely related Polyommatainae and Theclinae. Although Malicky (1970) concluded that the lack of a honey gland and eversible

tubercles in the Lycaeninae are derived features, he was apparently unaware of strongly myrmecophilic members of this group and of the relationship of dendritic setae to myrmecophily.

DeVries (1988, 1991b) concluded that riodinine ant-organs are analogous but not homologous to the ant-organs of other lycaenids, and that myrmecophily among riodinines was derived independently. DeVries (1991b) also questioned the importance of lenticles in myrmecophily, and rejected the notion that myrmecophily is ancestral in Lycaenidae. However, he was unaware that dendritic setae, which occur widely among myrmecophilous lycaenids (including riodinines), may represent not only the most primitive ant-organ (if lenticles are excluded) but a common precursor to eversible tubercles and anterior tentacle organs, as well. Nevertheless, because the presence of dendritic setae is apparently as labile as that of other ant-organs, and because the structure of dendritic setae may be a simple derivation of 'standard' body setae, the possibility of independent derivation in different lineages cannot be ignored.

Resolution of questions regarding lycaenid phylogeny may ultimately depend on an integrated analysis of many factors involving both immatures and adults. Because of broad diversity and lability of feeding habits, myrmecophily, and larval morphology within higher taxonomic groups, evolutionary patterns based on such features should be corroborated by comparison with ostensibly more stable characters, such as first instar chaetotaxy. Also valuable may be studies of the sites of origin and structures of semiochemicals related to myrmecophily and comparisons of DNA among representatives of the various taxonomic groups.

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